

The effect of extrusion conditions on the acidic polysaccharide, ginsenoside contents and antioxidant properties of extruded Korean red ginseng

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This study was conducted to investigate the effect of extrusion conditions (moisture content 20% and 30%, screw speed 200 and 250 rpm, barrel temperature 115°C and 130°C) on the acidic polysaccharide, ginsenoside contents and antioxidant properties of extruded Korean red ginseng (KRG). Extruded KRGs showed relatively higher amounts of acidic polysaccharide (6.80% to 9.34%) than non-extruded KRG (4.34%). Increased barrel temperature and screw speed significantly increased the content of acidic polysaccharide. The major ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf, Rg2s, Rg3s, Rh1, and Rg3r) of KRG increased through extrusion, while the ginsenoside (Rg1) decreased. The EX8 (moisture 30%, screw speed 250 rpm, and temperature 130°C) had more total phenolics and had a better scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radicals than those of extruded KRG samples. The extrusion cooking showed a significant increase (6.8% to 20.9%) in reducing power. Increased barrel temperature significantly increased the values of reducing power, the highest value was 1.152 obtained from EX4 (feed moisture 20%, screw speed 250 rpm, and temperature 130°C). These results suggest that extrusion conditions can be optimized to retain the health promoting compounds in KRG products.

Keywords: *Panax ginseng*, Korean red ginseng, Extrusion, Acidic polysaccharide, Antioxidant properties

INTRODUCTION

Ginseng (*Panax ginseng* Meyer) is a perennial plant that has been used as a medicinal plant and a functional food for more than 2000 years. It is found that ginseng contains a lot of biologically active ingredients like ginsenosides, acidic polysaccharides, polyacetylenes, peptides and phenolic compounds [1].

It is known that fresh ginseng once air-dried, yields white ginseng and when steamed between 90°C to 100°C for 2 to 3 h followed by drying under sunlight yields red ginseng. In comparison to fresh ginseng and white ginseng, red ginseng has a better storage stability. In addition, steaming and drying can improve the effec-

tive components of ginseng by inactivating catabolic enzymes and releasing the antioxidant substances from ginseng [2]. Baek et al. [3] also reported that a heat processing induced structural and chemical transformations leading to improved biological activities and effectiveness. Also, red ginseng has demonstrated more pharmacological effects than white ginseng; the ginsenoside Rg3 in red ginseng inhibits platelet aggregation and cancer cell metastasis and induces vasorelaxation [4]. However, it's difficult to extract the active components because of its denser texture. Recently, red ginseng has become an attractive ingredient in the extrusion industry due to its

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unique attributes such as dense texture and low yield of extraction.

Extrusion cooking is a continuous process which utilizes high temperature, pressure and shear force to produce a product with unique physical and chemical characteristic. Compared to traditional food processing systems, extrusion cooking improves digestibility and nutrients bioavailability. Product characteristics of extrudates depend on physicochemical changes that occur during the extrusion process. Critical extrusion process variables such as moisture content, screw speed and barrel temperature may induce desirable modifications, thus improve palatability and technological properties of extruded products. The previous study on the effects of extrusion conditions on the physicochemical properties of ginseng extrudates, Ha and Ryu [5] reported that acidic polysaccharide content was increased by 2% to 3%, and ginsenoside Rg3 was detected in extruded Korean red ginseng (KRG). Crude saponin and ginsenoside (Rg1 and Rg2) content also increased after extrusion cooking [6]. Additionally, Han et al. [7] found that the α -amylase susceptibility of ginseng put through the extrusion cooking has been found to be lower than that of traditionally dried ginseng. On the other hand, an antioxidant compound was found in extruded ginseng sample using TLC method. Ryu [8] demonstrated that extruded dry ginseng (moisture content 25%, screw speed 200 rpm, and barrel temperature 110°C) showed higher electron donation ability and phenolic content than those of ginseng samples.

The researches, so far, on the physicochemical properties of extruded KRG and the effect of extrusion variables on extruded KRG as a control, or it is produced by a single condition of extrusion. To our knowledge, this is the first report on the changes of acidic polysaccharide, ginsenosides contents and antioxidant properties of extruded red ginseng by different extrusion conditions. With the increased use of twin-screw extruders in the manufacture of products by adding extruded KRG extract (powder), it is necessary to have enough data on extrusion of KRG. Therefore, the objective of the present study was to investigate the effects of extrusion conditions (feed moisture content, screw speed, and barrel temperature) on effective components and antioxidant properties of an extruded KRG.

MATERIALS AND METHODS

Materials

The 5-year-old KRG (moisture content 9.50%, fat content 1.36%, protein content 14.57% and ash content

4.90%) was purchased in dried powder forms from the National Agricultural Cooperative Federation in Seosan, Korea. Standards of ginsenoside Rg1, Re, Rf, Rh1, Rg2s, Rg2r, Rb1, Rc, Rb2, Rd, Rg3s, Rg3r, Rh2r, and Rh2s were purchased from the ChromaDex (Seoul, Korea). HPLC-grade acetonitrile and methanol were purchased from Merck Co. (Merck, Darmstadt, Germany). Deionized water was purified using the Milli-Q system (Millipore, Bedford, MA, USA). Other reagents used in this study were of analytical grade.

Extrusion process

The red ginseng powders were extruded with a co-rotating intermeshing twin-screw extruder (THK31T; Incheon Machinery, Incheon, Korea) with a screw length of 768 mm and a screw diameter of 32 mm (L/D=24:1). A circular die (3.0 mm diameter) was used. The KRG powder feed rate was maintained at 120 g/min, and water was injected into the barrel around the feed section to adjust the feed moisture content at 20% and 30%. Extrusion was carried out at screw speeds of 200 and 250 rpm and the temperature of the extruder barrel was maintained at 115°C and 130°C. The extrudate was dried directly in an air oven at 60°C for 8 h, and ground in a laboratory grinder to pass through a 400 μ M mesh sieve, then stored in plastic bags for further analysis.

Acidic polysaccharide contents

The content of acidic polysaccharide was measured according to the carbazole-sulfuric acid method [9] as follows. Briefly, 0.5 mL of the sample extract solution was mixed with 0.25 mL of carbazole-absolute ethanol (0.1%, v/v) and 3 mL of concentrated sulfuric acid. Then the mixing solution was placed in 80°C water for 5 min and cooled at room temperature. The absorbance was read in a cuvette at 525 nm. D-galacturonic acid was used as a standard.

Ginsenosides analysis

Ginsenosides were determined using UPLC (Acquity UPLC System; Waters, Milford, MA, USA), equipped with a binary solvent delivery system, an autosampler and a tunable UV detector, and an Acquity UPLC BEH C₁₈ column (1.7 μ M, 2.1×100 mm) also from Waters was used. The samples (0.5 g) were dissolved in 10 mL of 50% methanol and were ultra-sonicated for 30 min, and then the mixtures were centrifuged at 1,000 g for 10 min. The injection volume was 5 μ L and the absorbance was measured at 203 nm. The two mobile phases were phase A: water-formic acid (100:0.2, v/v); phase B: acetonitrile

trile, and the UPLC elution conditions were optimized as follows: linear gradient from 5% to 15% B (0 to 5 min), 15% to 35% B (5 to 10 min), 35% to 42% B (10 to 14 min), and 42% to 80% B (14 to 20 min). The flow rate was set at 0.4 mL/min and the column temperature was maintained at 30°C.

Antioxidant properties

The ground KRG samples (1.0 g) were dissolved in 5 mL of distilled water and then 25 mL of ethanol-water (ethanol/water, 80:20, v/v) solution was added, and were extracted at 30°C for 4 h. The extracts were centrifuged at 1,000 g for 20 min and filtered through Whatman no.1 filter paper. The filtrates were transferred into a sample vial for total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and reducing power (RP) analyses.

Total phenolic contents

The total phenolic content (TPC) of sample solution was determined according to the method of Norajit et al. [10], with some modifications. An aliquot of the extract (0.1 mL) was mixed with 1.5 mL freshly prepared Folin-Ciocalteu reagent diluted with distilled water (10-fold). The mixture was allowed to equilibrate for 5 min and then 1.5 mL of 10% sodium carbonate was added. After incubation at room temperature for 90 min, the concentration of total phenolic contents was measured by reading absorbance at 765 nm, while the ethanol-water (ethanol/water, 80:20, v/v) was used as a blank. Gallic acid was used as the standard. Results in triplicate were expressed as mg of gallic acid equivalents (GAE) per gram of dry sample.

2,2-Diphenyl-1-picrylhydrazyl radical-scavenging activity

The hydrogen-donating or radical-scavenging of the sample solution was measured by the DPPH method proposed by Brand-Williams et al. [11]. A solution of DPPH (5.0 mL, 0.1 mM) in absolute ethanol was added to 0.5 mL of extract sample solution. The mixture was then incubated at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm. The ethanol substituted the sample solution was used as a control. For the comparison, the assay was conducted in the same manner but butylhydroxytoluene (BHT) was added instead of sample solution. Samples were assayed in triplicate. The capability of scavenging the DPPH radical was calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A_{control} is the absorbance of the control, and A_{sample} is the absorbance of the sample.

Reducing power assay

The RP of sample solutions was measured as described by Gulcin et al. [12]. The reaction mixture was composed of 1.0 mL of the sample solution, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. After incubation at 50°C for 20 min, then the 2.5 mL of 10% trichloroacetic acid was added to the mixture solution. After centrifugation at 1,000 g for 20 min, the supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of a 0.1% ferric chloride solution. The absorbance was recorded at 700 nm after 10 min. The absorbance value indicates the RP. For the comparison, the assay was conducted in the same manner but BHT was added instead of sample solution. Triplicate analyses were run for each extract.

Statistical analysis

ANOVA was carried out using SPSS 18.0 ver. (SPSS Inc., Chicago, IL, USA). All data were recorded as means±SD. Mean values were calculated according to Fisher's least significant difference test ($p < 0.05$).

RESULTS AND DISCUSSION

Acidic polysaccharide contents

Since the first research on ginseng polysaccharide reported by Ovodov and Solov'eva [13], 35 polysaccharides were identified from the leaves, roots and fruits of ginseng. Modern pharmacological studies had found that ginseng polysaccharides had immunomodulation, antitumor, antiadhesive, antioxidant activities, and so on [14]. Hence ginseng acidic polysaccharide has become an attractive chemical component in the ginseng research due to its biological and pathological activities.

In this study, the contents of acidic polysaccharide were also measured, and the results of which are shown in Fig. 1. Extruded KRG showed higher acidic polysaccharide contents (6.80% to 9.34%) than non-extruded KRG (4.34%), which means an increase of 1.57 to 2.15 times. Previous studies reported that the cell wall was present in red ginseng (raw material) but not in extruded red ginseng [8]. This was probably due to change of the cell wall structure as a result of shear force coming from screw rotation along with heating and applying pressure

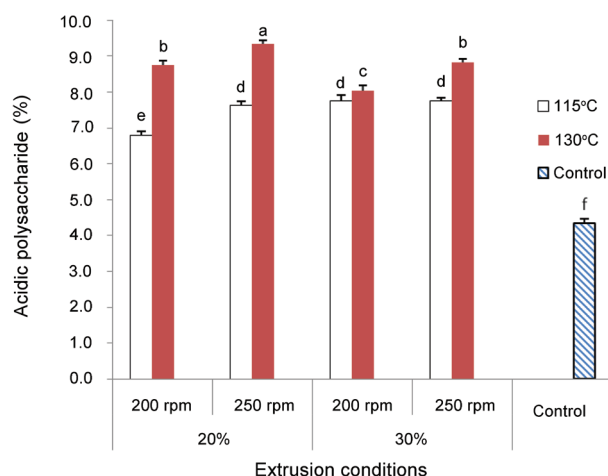


Fig. 1. Acidic polysaccharide contents (%) of control and extruded Korean red ginseng samples. Control: non-extruded red ginseng (raw material). Extrusion condition: moisture content 20% and 30%, screw speed 200 and 250 rpm, temperature 115°C and 130°C. Each bar represents the mean±SD. Different letter superscripts denote significant difference ($p < 0.05$).

inside the barrel. During the extrusion process, the extraction yield of the acidic polysaccharides increase due to cell wall damage when the products were put through the extruder. This result concurs with the finding [5] that the acidic polysaccharide content was increased by the extrusion process. On the other hand, barrel temperature and screw speed were observed to have a significant effect whereas moisture content was observed to have a slight impact on the acidic polysaccharide content of extrudates. In addition, from this research we concluded that increasing barrel temperature and screw speed both significantly increased the content of acidic polysaccharide. This result was in agreement with Yoon et al. [15], who also reported that the acidic polysaccharide contents

of ginseng increased with the increase in heating temperature and time.

Ginsenosides analysis

Ginsenosides are frequently used as main index for ginseng products evaluation. More than 30 ginsenosides are known, the most abundant components present in ginseng are Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, etc. In this study, the ginsenosides Rg1, Re, Rf, Rh1, Rg2(s), Rg2r, Rb1, Rc, Rb2, Rd, Rg3(s), Rg3r, and Rh2s were measured (Fig. 2). As shown in Table 1, the total ginsenoside content increased after extrusion, which means an increase of 1.26 to 1.37 times. The highest content was obtained at EX5 and EX7 (feed moisture 30%; temperature 115°C; and screw speed 200, 250 rpm; respectively). Ginsenoside Rd increased about 2 times, from 0.69 to 0.80 mg/g. This increase is probably due to heat energy and mechanical energy coming from shear force and pressure during the extrusion [5]. In addition, increased barrel temperature resulted in a slight decrease in ginsenosides (Re, Rf, Rb1, Rb2, Rc, and Rd). A previous study has already reported that heat degrades ginsenosides, especially the thermally unstable malonyl ginsenosides [16]. Subsequently, the increased feed moisture content increased the ginsenosides (Rb1 and Rg3) and screw speed was found to have a slight influence on the ginsenosides of extruded KRG. Ginsenoside Rg3 increased by about 1.3 times. Ginsenosides Rg3, which contains two glucose residues bound to C-3, is formed by eliminating the glycosyl residue at C-20 of many protopanaxadiol ginsenosides (Rb1, Rc, Rd, etc.) [4]. When the extrusion is applied to ginseng or red ginseng, some ginsenosides are hydrolyzed by heat, while high moisture content may have a protective effect [2].

Table 1. Ginsenoside contents (mg/g) of control (non-extruded) and extruded Korean red ginseng at different extrusion conditions

Extrusion conditions			Ginsenoside (mg/g)											Total
Moisture content (%)	Screw speed (rpm)	Temperature (°C)	Rg1	Re	Rf	Rh1	Rg2s	Rb1	Rc	Rb2	Rd	Rg3s	Rg3r	
	Control		3.05	2.07	0.92	0.30	0.37	6.48	2.10	1.95	0.42	0.36	0.18	18.19
20	200	115	3.01	2.87	0.98	0.27	0.50	8.83	3.31	3.12	0.75	0.42	0.20	24.26
		130	2.97	2.70	0.92	0.31	0.53	8.41	3.12	2.94	0.72	0.41	0.20	23.23
	250	115	2.94	2.75	0.95	0.26	0.42	8.45	3.14	2.95	0.69	0.41	0.19	23.15
		130	3.00	2.67	0.99	0.37	0.53	8.40	3.06	2.91	0.73	0.41	0.21	23.28
30	200	115	3.04	2.92	0.99	0.30	0.56	9.06	3.41	3.21	0.80	0.44	0.21	24.94
		130	2.98	2.65	0.95	0.32	0.48	8.33	3.01	2.87	0.70	0.46	0.25	23.01
	250	115	3.07	2.95	0.97	0.34	0.55	9.07	3.39	3.20	0.76	0.42	0.21	24.95
		130	2.82	2.63	0.94	0.39	0.56	8.28	3.14	2.90	0.72	0.47	0.25	23.11

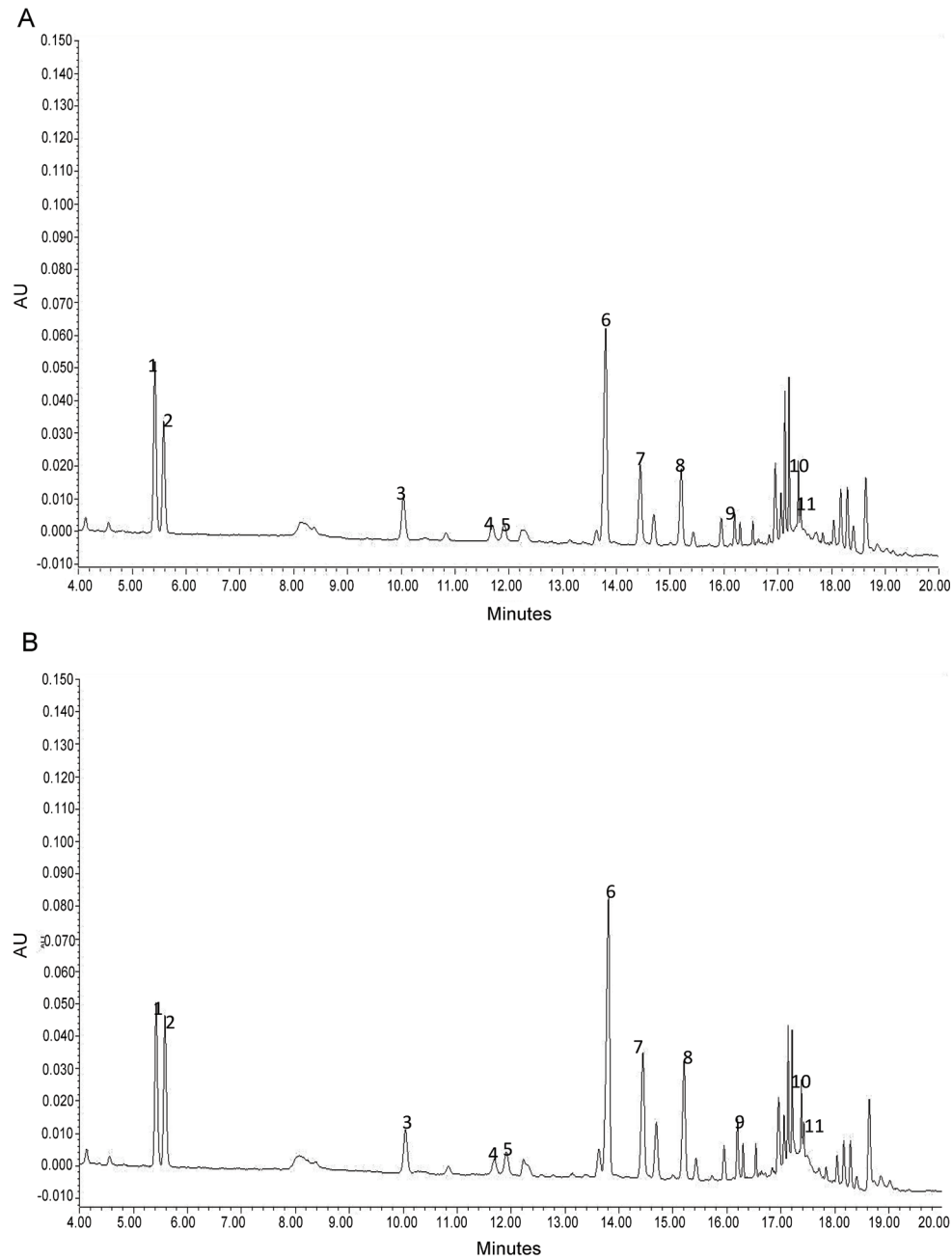


Fig. 2. UPLC chromatograms of (A) non-extruded (control) Korean red ginseng (KRG) and (B) extruded KRG (moisture content 30%, screw speed 250 rpm, and barrel temperature 115°C) red ginsengs. UPLC was performed on a BEH C_{18} (1.7 μ M, 2.1 \times 100 mm) at column temperature of 30°C. The two mobile phases were phase A: water-formic acid (100:0.2, v/v); phase B: acetonitrile, using the following gradient program: linear gradient from 5% to 15% B (0 to 5 min), 15% to 35% B (5 to 10 min), 35% to 42% B (10 to 14 min), and 42% to 80% B (14 to 20 min). The flow-rate was at 0.4 mL/min and the sample injection was 5 μ L. Peaks: 1, Rg1; 2, Re; 3, Rf; 4, Rh1; 5, Rg2s; 6, Rb1; 7, Rc; 8, Rb2; 9, Rd; 10, Rg3s; 11, Rg3r.

Antioxidant properties

Many methods are available to evaluate the different antioxidant activities of natural compounds from plant extracts and foods. The antioxidant properties of extruded KRG in this study were tested for their total phenolic contents, DPPH radical scavenging effect and RP.

Polyphenolic compounds are plant secondary metabolites of biological and pharmacological significance. The antioxidant activity of plant caused mainly by phenolic compounds has been demonstrated in many studies over recent years. As shown in Table 2, the TPC in the eight extrusion conditions ranged from 4.20 to 5.16 mgGAE/g.

Table 2. Total phenolics, radical-scavenging activity (DPPH) and reducing power (RP assay) of control (non-extruded) and extruded Korean red ginseng

Extrusion conditions			Total phenolics (mgGAE/g)	Antioxidant properties	
Moisture content (%)	Screw speed (rpm)	Temperature (°C)		DPPH (%) ¹⁾	RP ²⁾
	Control		4.73±0.02 ^d	59.99±0.57 ^e	0.953±0.006 ^e
20	200	115	4.51±0.05 ^e	62.32±0.47 ^d	1.048±0.008 ^e
		130	4.98±0.03 ^{bc}	67.81±0.32 ^c	1.074±0.003 ^e
		250	4.59±0.02 ^e	62.88±0.75 ^d	1.047±0.005 ^e
30	200	115	5.03±0.05 ^b	70.84±0.27 ^b	1.152±0.047 ^a
		130	4.20±0.10 ^f	58.64±0.47 ^f	1.052±0.012 ^c
		130	4.92±0.02 ^c	70.38±0.48 ^b	1.114±0.001 ^b
	250	115	4.21±0.04 ^f	58.99±0.47 ^f	1.018±0.005 ^d
		130	5.16±0.03 ^a	71.81±0.16 ^a	1.124±0.002 ^{ab}
	BHT ³⁾ (0.5 mg/mL)			80.20±0.51	1.418±0.015

Each value is expressed as mean±SD (n=3).

Different letter superscripts denote significant difference (p<0.05).

DPPH, 2,2-diphenyl-1-picrylhydrazyl; RP, reducing power; GAE, gallic acid equivalents; BHT, butylhydroxytoluene.

¹⁾ DPPH value expressed as % of dry sample.

²⁾ RP value expressed as absorbance at 700 nm.

³⁾ The concentration is indicated in the parenthesis.

Interestingly, compared with the non-extruded KRG (4.73 mgGAE/g), a lower value (4.20 to 4.59 mgGAE/g) and a higher value (4.92 to 5.16 mgGAE/g) were observed at 115°C and 130°C, respectively. There was a significant difference (p<0.05) between samples in terms of TPC for the products processing by tested extrusion conditions. With an increasing barrel temperature (115°C to 130°C), the TPC was significantly increased. However, increased feed moisture led to a slight decrease in TPC. Screw speed was observed to have no significant effect on the TPC. As we already studied, the extrusion process could cause damage of cell structures of plant and allowed easier extraction of soluble substances from samples itself, which increased the total phenolic compounds of extrudates. Shih et al. [17] also reported that the increase in the levels of phenolic acids (particularly ferulic acid) in extruded products is generally due to the release of acid and its derivatives from the cell walls of the plant matter. A more thorough study has been carried out by Zielinski et al. [18] and has revealed that the ferulic acid content may increase by three times due to extrusion. On the other hand, extrusion of common beans with corn starch blend is reported to increase total phenol and antioxidant potential of extruded snacks [19]. They found a significant increase in the total phenol content of extruded snacks obtained from blends of corn starch and small red beans. This may be attributed to the color of samples. Therefore, we think that the increase of TPC due to high barrel temperature and low feed moisture content may

increase the redness of extrudates. In this study, the decrease of TPC is probably caused by an interaction effect of feed moisture and barrel temperature.

DPPH radical is a widely used assay to evaluate antioxidant activities in a relatively short time compared to other methods. The color changes from purple to yellow by acceptance of an electron or hydrogen radical from an antioxidant and it becomes a stable molecule. The DPPH radical scavenging activities of samples with tests extrusion conditions are shown in Table 2. The results showed that, compared with the non-extruded KRG (59.99%), the DPPH radical scavenging activities decreased (p<0.05) in EX5 and EX7 (feed moisture 30%, temperature 115°C, and screw speed 200 and 250 rpm, respectively) by 2.3% and 1.7%, respectively while the rest of the conditions did show significant (p<0.05) increase (3.9% to 19.7%). This may be attributed to the browning reaction induced by the extrusion process. The earlier investigations have reported that dark color pigments (brown color) are produced during the thermal processing of foods due to the Maillard browning [20]. These pigments (particularly melanoidins) are extensively known to have antioxidant activity [21], and other studies also have demonstrated that thermal processing may increase the antioxidant activity. Similar findings have been reported in the literature [22,23]. It is found that extracts from extruded cereal grain or cereal bran exhibited a greater antioxidant activity than did those from non-extruded grain or bran. Furthermore, the DPPH

radical scavenging activities increased ($p < 0.05$) substantially with increasing barrel temperature. When the barrel temperature was kept constant (130°C), increased screw speed and feed moisture content both significantly increased the antioxidant activities. Additionally, when the barrel temperature was kept constant (115°C), increased feed moisture content led to a slight decrease in the antioxidant activities. The EX8 (feed moisture 30%, screw speed 250 rpm, and temperature 130°C) showed the highest antioxidant activity. Delgado-Licon et al. [24] investigated the influence of extrusion on the bioactive compounds and the antioxidant capacity of the bean/corn mixtures, and found that the highest antioxidant activity of extruded bean/corn mixture was obtained at 142°C barrel temperature. A change in the extrusion parameters (including barrel temperature, screw speed, moisture content and raw material used) could have led to the formation of different amounts of Maillard browning product [21]. Additionally, Oomah et al. [25] reported that the antioxidant activity is not only affected by quantity but also the kind of free radical scavengers present in the material.

The RP is often used as an indicator of electron donating activity, which is an important mechanism for testing the antioxidant activity. The extrusion cooking showed a significant increase (6.80% to 20.9%) in RP (Table 2). The RP of samples is mainly due to the phenolic compounds as the phenolic compounds have the ability to donate electrons and play a major role in the RP. Therefore, the increase in TPC may be a reason for the increase in RP after extrusion. A similar increase in RP has been reported by other authors upon the roasting process in oats [26]. In addition, an increase of barrel temperature used in extrusion cooking resulted in increased RP. Feed moisture content and screw speed had a slight influence on the RP. It is already reported that the soluble fraction of the Maillard reaction products contributes to the RP [27]. Hence, it may be possible that the amount of soluble fraction of Maillard reaction products was also affected on extrusion at different conditions.

Extrusion cooking exhibited a significant effect on the antioxidant properties of extruded KRG. In all extruded samples, total phenolic levels were significantly ($p < 0.05$) correlated to both DPPH ($r^2 = 0.8317$) and RP ($r^2 = 0.8336$) antioxidant activities. Overall, the extruded KRG revealed better antioxidant properties than the non-extruded KRG. Ryu [8] also reported that extruded ginseng has a better antioxidant effect than their corresponding control samples and it can be created by controlling the extrusion parameters.

In this work, the effects of variable moisture content, screw speed and barrel temperature on the acidic poly-

saccharide, ginsenoside contents and antioxidant properties of red ginseng powder extrudates were investigated. After extrusion, the acidic polysaccharide and total ginsenoside contents were significantly increased. Increased barrel temperature and screw speed resulted in increased acidic polysaccharide and total phenolic contents. Using the DPPH radical-scavenging assay, EX8 (moisture 30%, screw speed 250 rpm, and temperature 130°C) had the highest antioxidant activity, but when using the RP assay, EX4 (moisture 20%, screw speed 250 rpm, and temperature 130°C) had the highest RP value. Increased barrel temperature resulted in obviously increased DPPH radical-scavenging activity and the RP value. These results will be used to help define an optimized process condition for controlling and predicting qualities and characteristics of extruded red ginseng.

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